The Influence of Iron on the Cellular Quota of Prochlorococcus

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LONG-TERM GOALS

Factors that control the distribution of marine phytoplankton are critical to our understanding of the primary productivity and global carbon cycling. In addition, the pigmentation of phytoplankton and the exudates of phytoplankton (colored dissolved organic matter) are two of the major factors influencing ocean optical properties. The cyanobacteria, including *Prochlorococococcus marinus*, are among the most abundant phytoplankton in the oceans, and hence contribute to both carbon cycling and ocean optical properties. Through this ONR-YIP grant, our laboratory has been studying an important component relating to growth of *Prochlorococcus*: the cellular iron quota. Because *Prochlorococcus* is relatively difficult to grow and cultures free of bacterial contaminants were only recently produced, studies of the elemental composition of this key phytoplankter have not been possible until very recently. Yet, global ecosystem modelers require iron quotas for all key phytoplankton groups in order to improve our predictive ability of primary productivity and the seawater optical properties throughout the oceans.

OBJECTIVES

There are five major objectives in this ONR-YIP project. First, we plan to measure the iron requirement of *Prochlorococcus* through growth rate experiments. Second, we plan to quantify the iron cellular quota under a range of iron and light conditions. Third, we will quantify colored dissolved organic matter production from iron limited cultures. Fourth, we will examine the physiology of *Prochlorococcus* under iron limitation. Fifth, we will examine the distribution of *Prochlorococcus* in field populations for comparison with iron concentrations.

APPROACH

We have undertaken careful physiological studies of iron growth requirements of representative *Prochlorococcus* strains and made concurrent measurements of their cellular iron quotas using inductively coupled plasma mass spectrometry (ICP-MS). Growth studies are being performed using cultures of *Prochlorococcus* and media prepared in a trace metal cleanroom, which prevents dust particles from contaminating cultures and thus allowing the metal limitation conditions needed for experiments. To complement these studies, analyses of the whole genomic response of *Prochlorococcus* under iron stress has been undertaken using microarray technology. This work has largely been being carried out by graduate student Anne Thompson, a MIT-WHOI Joint Program student in Biology, co-advised by Saito and Sallie Chisholm (MIT). Alysia Cox is a MIT-WHOI Joint

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Program student in Marine Chemistry and Geochemistry who is also working on ancillary studies of cadmium utilization by marine cyanobacteria.

WORK COMPLETED

Growth rate experiments were completed on *Prochlorococcus* strain MED4 and MIT9313. Samples were taken for CDOM analyses and Fe cellular quotas. In year 2, the cellular quota method was further developed to allow for lower blanks and increased sensitivity. Microarray analyses have now been completed on two strains of *Prochlorococcus* MED4 and MIT9313, and using abrupt iron limitation as well as steady state iron limitation experiments. Field samples from a R/V Knorr cruise in the summer of 2005 are being analyzed for *Prochlorococcus* and iron concentrations. Method development for the iron concentration analyses was completed and published (Saito and Schnieder, 2006). Cadmium uptake methodologies were developed using stable isotope additions and inductively coupled plasma mass spectrometry. Finally a synthesis manuscript on the colimitation of marine phytoplankton by multiple nutrients was completed (Saito et al., in press).

RESULTS

At the end of our second year of this ONR-YIP project we have continued to make significant progress on the objectives outlined above (see Objectives section). In particular the first through fourth objectives have largely been completed, or have samples waiting to be analyzed in the near future (e.g. #3). Data analysis and finalizing data sets remain the primary objectives in these goals. Analysis of field samples (#5) is currently underway. In particular analysis of iron distributions with respect to *Prochlorococcus* populations is underway, with the latter largely completed and the former under analysis. There have also been several additional projects undertaken that contribute directly to the overall goals of this ONR proposal, specifically including an examination of cadmium uptake and toxicity as well as a synthesis of the concept of colimitation in marine phytoplankton. These results of the above mentioned projects are addressed in the following paragraphs.

Culturing experiments on the iron growth requirements of *Prochlorococcus* and concurrent measurements of cellular iron quotas using inductively coupled plasma mass spectrometry (ICP-MS) have yielded several important insights thus far. First, physiological experiments show that iron requirements of *Prochlorococcus* appear to be lower than the estimated quantities of available “free” iron in seawater (Figure 1). In seawater, iron is complexed by strong organic ligands, and the iron bound by those ligands is believed to be harder to acquire for phytoplankton than the “free” forms of iron. This implies that *Prochlorococcus* must be able to utilize the ligand-bound iron in order to maintain their growth rates. This is an important fact that has broad implications for our understanding of *Prochlorococcus* growth and iron cycling in the oceans. Second, our preliminary cellular iron quota measurements suggest that there is an effect of light on the content of iron within the cell, consistent with the high iron requirement of the Photosystem I apparatus. Third, whole genome microarray studies demonstrate a number of genes that are affected by iron limitation in the *Prochlorococcus* strain MED4 (Figure 2). Knowing the identity of genes that are influenced by iron is important for several reasons: first, they assist in understanding how the physiology of *Prochlorococcus* is affected by iron nutrition; second, they provide crucial information about candidate genes that can be utilized as biomarkers of iron stress. Many of these genes that respond to iron limitation do not currently have a known function, when compared to current genomic and biochemical knowledge. As a result, they may be genes that are involved in the utilization of the ligand-bound iron described above.

Understanding how the cyanobacteria acquire their iron is currently a mystery in the oceans, and examination of these genes might provide significant insight into this important process. In addition to the perturbation experiment described above, new steady state iron microarray experiments have been conducted on two strains of *Prochlorococcus*, and show a complex response of many genes. Proteomic samples have also been taken of these experiments to allow transcriptome-proteome comparisons.

The interaction of iron and light is believed to be one of the most important "colimitations" that exist in the natural environment. The term colimitation refers to instances where two nutrients are limiting simultaneously. However, significant confusion exists about the meaning of the term colimitation. We have written a synthesis manuscript this year that defines three types of colimitation based on biochemical relationships: independent, biochemical substitution, and biochemical dependence (Saito et al., in press for 2008). These colimitations were described in terms of modified Monod-type equations and represented visually as three dimensional images (Figure 3). Iron-light colimitation is a complex scenario since iron has long been known to be important in light acquisition in particular in the iron-rich photosystem I complex. Our microarray results with *Prochlorococcus* are consistent with this interpretation, where numerous genes involved in the Photosystem I complex were observed to be down-regulated during iron depravation, and then to be upregulated upon iron addition (Figure 4). Because of this functional use of iron in light acquisition we have defined iron on light colimitation as a Type III biochemically dependent system. Interestingly, this is significantly different than the current parameterizations of Type I biochemically independent used in global ecosystem models, and reflects the need for the modeling community to correct their colimitation algorithms to accurately represent the biochemistry of iron-light colimitation.

In the coming year, we plan to write up the *Prochlorococcus* physiological and molecular/microarray studies, as well as completely the remaining studies and analysis of samples. Additionally, we hope to begin to analyze the proteins being expressed under iron limitation as a validation of the genomic studies completed thus far, and as a means for verifying ideal candidates for the mass spectrometry iron stress assay discussed above. The results described above have already been presented at international meetings (Summer ASLO, 2006; Winter ASLO 2007). In summary, this study is the first comprehensive examination of iron and the marine cyanobacterium *Prochlorococcus* and has provided important information about the iron requirements, iron acquisition systems, gene regulatory response related to iron stress, and the parameterization of iron-light colimitation in ecosystem models. We anticipate that this information will be useful in understanding and predicting how iron distributions can affect the abundances and productivity of *Prochlorococcus*.

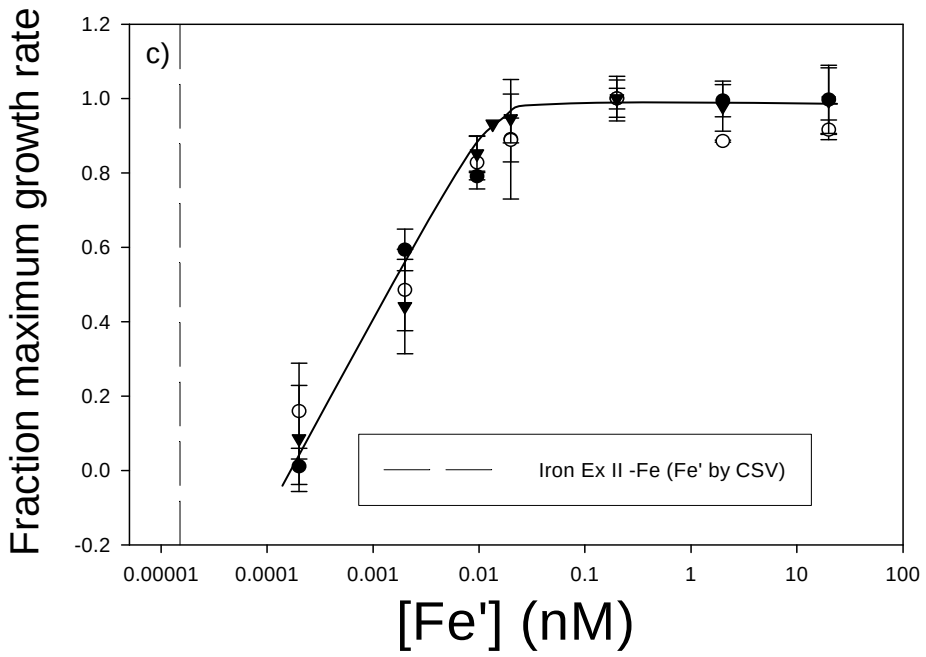

Figure 1. Laboratory experiments demonstrating the limitation of Prochlorococcus growth by iron. Fe' is representative of the "free" iron in seawater, and refers to the concentration of all inorganic species of iron (including free ions, Fe^{3+}, and hydroxo and chloro complexes). The vertical dashed line indicates the concentration of Fe' measured in the oceans by electrochemical techniques. Comparison of these two results clearly demonstrates that Prochlorococcus cannot maintain reasonable growth rates if only Fe' is available. Hence this data set is strong evidence for the utilization of the organically complexed iron in seawater, which exists in much higher concentrations. The genetic/chemical mechanism for removal of iron from these complexes by cyanobacteria is currently unknown, but genomic experiments should shed useful insight into these mechanisms (e.g. Figure 2). [Graph of fraction of maximum growth rate versus Fe', where fraction of maximum growth rate is ~1 between 30nM down to 0.1nM and begins to decrease at ~0.01nM and resulting in little to no growth below 0.001nM. The dashed line representing Fe' in the Pacific is much lower than the lowest Fe' needed to support Prochlorococus growth, indicative of the use of organically complexed iron.]

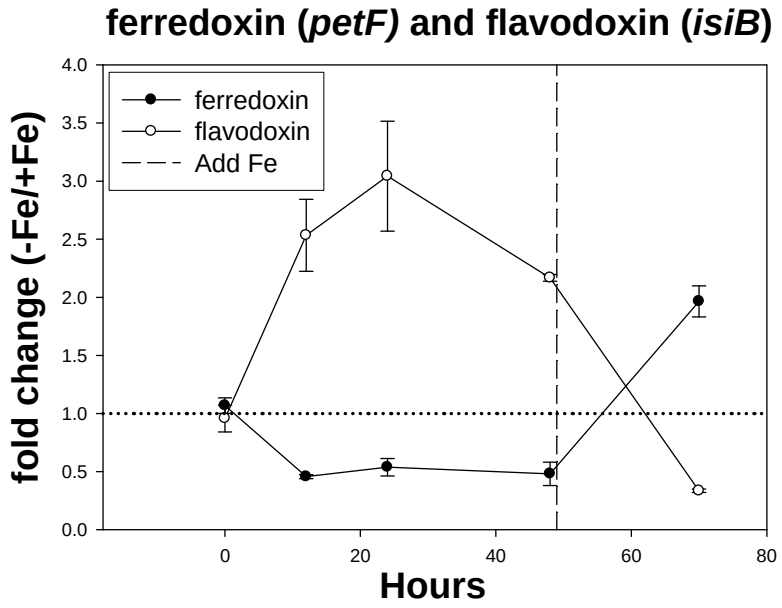

Figure 2. Whole Genome Microarray data for Prochlorococcus MED4 demonstrating increased flavodoxin transcripts (which does not contain iron) and decreased ferrodoxin transcripts (which contains iron) under iron stress. Upon iron addition (vertical dashed lines), transcripts signaling the production of each compound reverse, demonstrating a clear iron effect on these genes. Many other genes were also found to respond to iron stress and additions. [Graph displaying "fold change of the iron deplete treatment relative to iron replete treatment" relative to time (70 hour experiment), where ferredoxin is at ~0.5 and flavodoxin goes as high as 3.0. When iron is added at 50h to the –Fe treatment the trend reverses where ferredoxin increases to ~2 and flavodoxin decreases to ~0.5.]

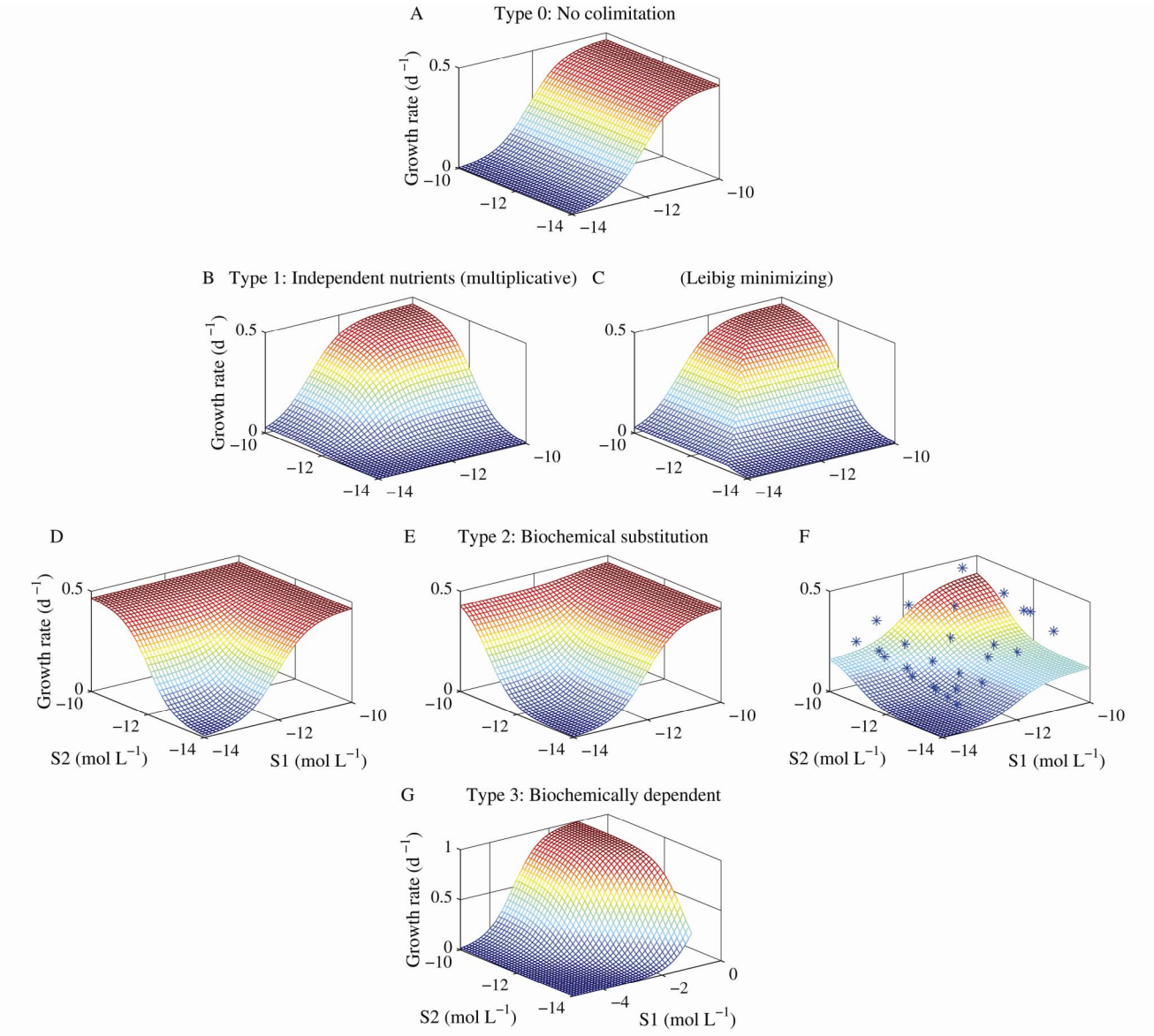

Figure 3. Three-dimensional representations of colimitation scenarios for marine phytoplankton, where S_1 and S_2 are the substrate nutrients. The concept of colimitation has not been rigorously defined until this study, and iron-light colimitation is likely the most important colimitation in the marine environment. (A) Type 0 no colimitation concerns two elements, where only one is a nutrient. Type I independent nutrients concerns two nutrients that do not share a biochemical function, such as nitrogen and phosphorus. Two expressions of Type I are plotted: (B) the multiplicative form, and (C) the minimization (Liebig) form. Type II biochemical substitution concerns two micronutrients that can substitute for the same biochemical function, usually due to a metalloenzyme that can be active with two different metals (e.g., Zn and Co). Three scenarios are presented, (D) where two nutrients substitute perfectly for each other, (E) where the two nutrients have unique half saturation constants, and (F) where two nutrients only partially substitute for each other, leaving a non-substitutable component of each biochemical quota. In this case, maximal growth occurs when both nutrients are present, representing a situation where the cambialistic

metalloenzyme constitutes only a fraction of the total S_1 and S_2 quotas. (G) Type III biochemically dependent colimitation concerns two nutrients where the acquisition of one (S_1) is dependent on the sufficient nutrition of the other (S_2) (e.g., C and Zn or Fe and light). Iron and light limitation are believed to be biochemically dependent (Type III), due to the significant number of iron atoms found within the enzymes of photosystem I. Our data from Prochlorococcus microarray studies demonstrate the response of photosystem I to iron limitation, consistent with this assignment of Type III for iron and light. Importantly, ecosystem models instead use the Type I multiplicative colimitation parameterization (B) for iron-light colimitation, and likely should be changed to reflect these findings. From Saito et al., Limnol. Oceanogr. In press - January 2008.

[Three dimensional graphs with a surface that represent examples of each of the four types of colimitation in marine phytoplankton (Type 0, I, II, and III). Each type has a unique surface that relates to the biochemical nature of the colimitation. Growth rate is in the vertical axis, and substrate 1 and 2 (S1 and S2) are in the x and y axes. In Type 0 growth rate is high when S1 is high and does not respond to variations in S2. In Type I growth rate is highest when both S1 and S2 are high. Growth rate is low only when either S1 or S2 are low. In Type II growth is high when both S1 and S2 are high, and when either S1 and S2 is high. Growth rate is low only when both S1 and S2 are low. In Type III growth rate is high when both S1 and S2 are high, and low when either S1 or S2 is low. But in this case, more S1 is needed when S2 is low due to the biochemical dependence effect of S1 acquisition on S2.]

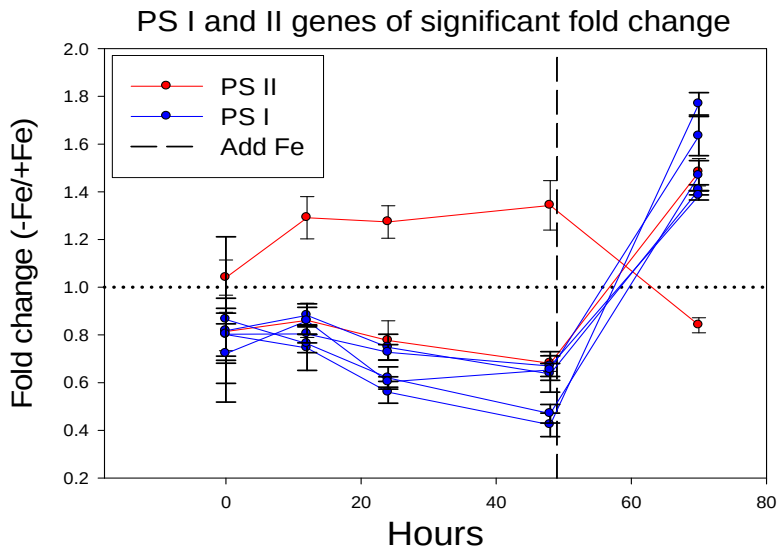

Figure 4. Microarray results demonstrating significant down-regulation of photosystem I genes during iron stress (0-47h), and up-regulation of those genes upon iron addition (dashed line). These results are consistent with the connection of the high iron requirement in photosystem I in Prochlorococcus. Given the fundamental importance of photosystem I to light acquisition and its clear response to iron limitation shown here, we have argued that iron-light colimitation is best described by Type III biochemically dependent algorithms as shown in Figure 3.

[Graph similar to that shown in Figure 2 with Fold change (-Fe/+Fe) relative to time in a 70h experiment. A number photosystem I genes are down-regulated clustering at ~0.8 and decreasing to a cluster around ~0.6, then increase to ~1.4-1.8 after the addition of iron to the –Fe treatment.]

IMPACT/APPLICATIONS

We anticipate that our iron cellular quota data for *Prochlorococcus* will be utilized in the ecosystem model components of recent coupled Ecosystem-Global Circulation Models (MOORE et al., 2002; MOORE et al., 2004). In addition, our synthesis of colimitation should be useful in the development of more realistic iron-light colimitation models in those ecosystems models. These models have the potential to generate predication of ocean optical properties based on predicted phytoplankton biomass and nutrient limitation patterns in the oceans.

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PUBLICATIONS

E.M. Bertrand*, **M.A. Saito***, J.M. Rose, C.R. Riesselman, M.C. Lohan, A.E. Noble, P.A. Lee, G.R. DiTullio. 2007. Vitamin B_{12} and iron co-limitation of phytoplankton growth in the Ross Sea. *Limnology and Oceanography*. 52:1079-1093. * co-first authors [published, refereed]

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HONORS/AWARDS/PRIZES

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